Eur J Nutr (2012) 51:1011–1019
DOI 10.1007/s00394-011-0280-6

ORIGINAL CONTRIBUTION

Silk and silkworm pupa peptides suppress adipogenesis in preadipocytes and fat accumulation in rats fed a high-fat diet

Sun Hee Lee · Dongsun Park · Goeun Yang · Dae-Kwon Bae · Yun-Hui Yang · Tae Kyun Kim · Dajeong Kim · Jangbeen Kyung · Sungho Yeon · Kyo Chul Koo · Jeong-Yong Lee · Seock-Yeon Hwang · Seong Soo Joo · Yun-Bae Kim

Received: 21 July 2011 / Accepted: 14 November 2011 / Published online: 13 December 2011

Abstract
Purpose The objective was to confirm the anti-obesity activity of a silk peptide (SP) and a silkworm pupa peptide (SPP) in rats fed a high-fat diet (HFD) and to elucidate their action mechanism(s) in a preadipocyte culture system.
Methods In an in vitro mechanistic study, the differentiation and maturation of 3T3-L1 preadipocytes were stimulated with insulin (5 μg/mL), and effects of SP and SPP on the adipogenesis of mature adipocytes were assessed. In an in vivo anti-obesity study, male C57BL/6 mice were fed an HFD containing SP or SPP (0.3, 1.0, or 3.0%) for 8 weeks, and blood and tissue parameters of obesity were analyzed.
Results Hormonal stimulation of preadipocytes led to a 50–70% increase in adipogenesis. Polymerase chain reaction and Western blot analyses revealed increases in adipogenesis-specific genes (leptin and Acrp30) and proteins (peroxisome proliferator-activated receptor-γ and Acrp30). The hormone-induced adipogenesis and activated gene expression was substantially inhibited by treatment with SP and SPP (1–50 μg/mL). The HFD markedly increased body weight gain by increasing the weight of epididymal and mesenteric fat. Body and fat weights were significantly reduced by SP and SPP, in which decreases in the area of abdominal adipose tissue and the size of epididymal adipocytes were confirmed by magnetic resonance imaging and microscopic examination, respectively. Long-term HFD caused hepatic lipid accumulation and increased blood triglycerides and cholesterol, in addition to their regulatory factors Acrp30 and leptin. However, SP and SPP recovered the concentrations of Acrp30 and leptin, and attenuated steatosis.
Conclusions SP and SPP inhibit the differentiation of preadipocytes and adipogenesis by modulating signal transduction pathways and improve HFD-induced obesity by reducing lipid accumulation and the size of adipocytes.

Keywords Silk peptide · Silkworm pupa peptide · Adipogenesis · Obesity · Hyperlipidemia · Steatosis

Sun Hee Lee and Dongsun Park, equally contributed to this work.

Electronic supplementary material The online version of this article (doi:10.1007/s00394-011-0280-6) contains supplementary material, which is available to authorized users.

S. H. Lee · D. Park · G. Yang · D.-K. Bae · Y.-H. Yang · T. K. Kim · D. Kim · J. Kyung · Y.-B. Kim (✉)
College of Veterinary Medicine and Research Institute of Veterinary Medicine, Chungbuk National University, 52 Naesudongro (Gaesin-dong), Cheongju, Chungbuk 361-763, Korea
e-mail: solar93@cbu.ac.kr

S. Yeon
Department of Food Science and Technology, Chungbuk National University, Cheongju, Korea

K. C. Koo · J.-Y. Lee
Worldway Co., Ltd., Jeoneui, Korea

S.-Y. Hwang
Department of Biomedical Laboratory Science, Daejeon University, Daejeon, Korea

S. S. Joo (✉)
Division of Marine Molecular Biotechnology, Gangneung-Wonju National University, 7 Jukheongil, Gangneung, Gangwon 210-702, Korea
e-mail: ssj66@gwnu.ac.kr

Introduction

Obesity, characterized as an excessive accumulation of fat in subcutaneous tissues or in abdominal cavity, has genetic or environmental causes, and its prevalence is steeply

increasing in developed countries [1]. Obesity is very influential for various diseases including cardiovascular disorders [2, 3]. High-energy intake-mediated obesity induces radical reactions in various organs and tissues, which cause tissue injury and dysfunction [4, 5]. In addition, obesity worsens the effects of diabetes mellitus in 80% of obese diabetics, and diabetes correlates with abdominal obesity, with morbidity risk increasing by 40-fold and 60.9-fold in persons with a body mass index of 35 and >35 kg/m^2, respectively. Furthermore, 60% of obese individuals experience health complications due to hypertension [6].

Although many risk factors of obesity include genetic background, endocrine dysfunction, metabolic disorders, low physical activity, inappropriate dietary habituation, and stresses, excessive net intake of calories (energy) is the major cause [7]. Interestingly, feeding pattern affects the metabolic rate of lipids; for example, ad libitum or meal feedings lead to different accumulation rates of triglycerides (TG) in adipocytes by altering the adipogenesis and adipolysis processes [8]. Especially, as TG synthesis occurs in mature adipocytes, the differentiation and maturation of preadipocytes to adipocytes are late-limiting factors for adipogenesis and obesity. During lipid metabolism, very low-density lipoproteins containing a high concentration of TG are hydrolyzed by lipoprotein lipase (LPL) to intermediate-density lipoproteins and low-density lipoproteins (LDL), which are removed by hepatic LDL receptors [9]. Insulin resistance accompanying obesity inhibits LPL, causing abnormal lipid metabolism in type II diabetes [10]. In spite of diverse factors inducing obesity, a high-fat diet (HFD) is one of the most important causes [11].

In animals and humans, HFD increases adipose tissue in the body, which has been considered to be suitable for studies on weight control and mechanisms of obesity as a dietary obesity model [11–13]. Unexpectedly in several rat model studies, an HFD containing 20–40% fat caused reduced feed intake, in addition to transient diarrhea, leading to decreased body weight of rodents fed ad libitum [14, 15]. C57BL/6 mice are a suitable model for induction of dietary obesity and hyperlipidemia, resulting in increased body weight [10, 16, 17]. D12451 and AIN94G are two commercially available HFDs that contain lard and shortening as fat sources, respectively [18, 19]. In the present study, C57BL/6 mice were fed D12451 containing candidate anti-obesity compounds, since D12451 induces high fat accumulation and body weight gain of the animals in a relatively short time.

Silk and silkworm pupa proteins have been ingested for a long time, without adverse effects, in Asian countries. Powder and extracts of silkworm (*Bombyx mori*) as well as silk proteins have diverse pharmacological activities, including improvement of hyperglycemia [20–22]. Also, silk peptides (SP) produced by enzymatic degradation of silk proteins regulate blood glucose and hyperlipidemia [23]. In addition to antioxidant effect, SP not only lowers blood cholesterol and TG, but also increases high-density lipoprotein [21, 24, 25]. Notably, silk and silkworm pupa amino acids reduced the body weight gain of resting and exercising animals [26, 27], implying that silk and silkworm pupa proteins and their degradation products affect lipid metabolism and body fat accumulation. It is of interest to note that silk and silkworm pupa amino acids, as nutrients, rather increased stamina in spite of their body weight-controlling activity [26, 27]. Such results led us to confirm the anti-obesity activity of SP and silkworm pupa peptide (SPP), which were prepared by treating silk and silkworm proteins, respectively, in a dietary obesity model and to reveal the action mechanism(s) in vitro cultured preadipocytes.

Materials and methods

Materials

SP and SPP (Worldway Co., Jeoneui, Korea) were attained by enzymatic degradation of silk proteins and silkworm pupa proteins by 0.5% Protease NP (Bioland, Chaonan, Korea) and 0.5% Protease N (Amano, Nagoya, Japan) plus 0.5% Alcalase (Novozymes, Bagsvaerd, Denmark), respectively, at 37 °C for 3 h, followed by lyophilization.

Cell culture and cytotoxicity

3T3-L1 preadipocytes (American Type Culture Collection, Manassas, VA, USA) were cultured in Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum [28]. The cells were seeded in wells of a 96-well plate (1×10^6 cells/mL), treated with SP or SPP (0.01–1,000 μg/mL), and incubated at 37 °C for 24 h. Cytotoxicity was assessed using a Cell Counting Kit-8 (CCK-8; Dojindo, Kumamoto, Japan) [29].

Differentiation of preadipocytes and adipogenesis

3T3-L1 cells in wells of a 96-well plate (1×10^6 cells/mL) were treated with SP or SPP (1–50 μg/mL). Differentiation was stimulated with a hormone sensitizer composed of insulin (5 μg/mL), dexamethasone (0.25 μM), and isobutyl methylxanthine (IBMX 0.5 mM) for 2 days, followed by insulin alone for additional 4 days to stimulate maturation [28]. The cells were fixed in a phosphate buffer containing 7% formaldehyde for 1 h and stained with 99% isopropanol containing 1% Oil red O for 10 min. The fat synthesized was quantified with an Adipogenesis Kit (#10006908;

Cayman, Ann Arbor, MI, USA) at 490 nm after extracting the Oil red O from the cells with isopropanol [28].

Gene expression and protein production

3T3-L1 cells in wells of a 6-well plate (1×10^6 cells/mL) were treated with SP or SPP (1–50 μg/mL) and insulin for differentiation and maturation, and then mRNA expression of adipogenesis-related genes, leptin and Acrp30 (adiponectin), was analyzed by reverse transcriptase-polymerase chain reaction (RT-PCR) with optimal primers (Bioneer, Daejeon, Korea) (Table 1). Production of peroxisome proliferator-activated receptor-γ (PPAR-γ) and Acrp30 protein related to adipogenic cell signaling was ascertained by Western blotting [28].

Animals and treatment

Six-week-old male C57BL/6 mice (mean body weight 20 g; Daehan Biolink, Eumseong, Korea) were housed in an environmentally controlled room with a temperature of 23 ± 2 °C, relative humidity of 55 ± 5%, and an alternating 12-h light (300 lux)/dark cycle, with pellet feed and purified water available ad libitum.

SP and SPP were mixed in powdered HFD (D12451; Research Diets Inc., New Brunswick, NJ, USA) containing 20.69% lard. The portions of casein matching the amount (0.3, 1.0, or 3.0%) of SP or SPP were removed (Supplementary Table 1). Control animals received a basal diet of D12450B for 8 weeks ad libitum. Study protocols met the guidelines of the Institutional Animal Care and Use Committee (IACUC) of Laboratory Animal Center at Chungbuk National University, Korea.

Feed intake and body and fat weights

Daily feed intake was recorded once a week for 8 weeks after providing with HFD containing peptides. Feed consumption was calculated by subtracting the remaining feed from the amount provided 24 h ago. Body weight of animals was recorded everyday at 10:00, and epididymal and mesenteric adipose tissues were weighed after killing following the 8-week treatment.

Size and distribution of adipocytes

Before killing, the mice were examined using magnetic resonance imaging (MRI), and the distribution of adipose tissue in abdominal transverse and coronal sections was determined [30]. After killing, epididymal fat tissue was fixed in 4% formaldehyde, and paraffin-embedded sections were stained with hematoxylin-eosin. Using a light microscopy (200× magnification), the mean size of 10 adipocytes was measured using an image analyzer.

Hepatic lipid accumulation

Liver tissue sections were stained with hematoxylin-eosin and examined for the lipid accumulation under a light microscope.

Serum biochemical analyses

Serum was analyzed for TG, total cholesterol, and glucose using a model 7180 blood chemistry analyzer (Hitachi Medical Co., Tokyo, Japan). Serum leptin and Acrp30 concentrations were measured with radioimmunoassay (RIA) using an animal leptin RIA kit (LINCO Research Inc., New York, NY, USA) and a mouse adiponectin RIA kit (Millipore, Billerica, MA, USA), respectively [10, 31, 32].

Statistical analyses

The results are presented as mean ± standard error and the significance of difference was analyzed using one-way analysis of variance followed by Tukey's test at the level of $P < 0.05$. The analyses utilized SPSS statistical software version 13.0 for Windows (SPSS, Inc., Chicago, IL, USA).

Results

Cytotoxicity of SP and SPP

In an in vitro CCK-8 test, SP exerted 20, 22, and 52% cytotoxicity on 3T3-L1 preadipocyte populations at concentrations of 10, 100, and 1,000 μg/mL, respectively (data

Table 1 Primer sequences for real-time RT-PCR

Gene	Primer	Amino acid sequences	Product size (bp)	Accession No.
Acrp30	Forward	5′-cgtcaatctcagcacttggg-3′	279	AF304466
	Reverse	5′-atgtcttgaggctaggcggt-3′		
Leptin	Forward	5′-agctgcaaggtgcaagaaga-3′	192	NM_008493
	Reverse	5′-accgactgcgtgtgtgaaat-3′		
β-Actin	Forward	5′-ctaggcaccagggtgtgatg-3′	291	NM_007393
	Reverse	5′-ctacgtacatggctggggtg-3′		

not shown). By comparison, SPP exhibited only 15 and 32% mortality at 100 and 1,000 μg/mL, respectively. Therefore, SP and SPP concentrations <100 μg/mL were subsequently used in experiments concerning preadipocyte differentiation, maturation, and adipogenesis.

Effects on preadipocyte differentiation and adipogenesis

Treatment of 3T3-L1 preadipocytes with a hormone sensitizer (insulin) increased adipogenesis by 50–70% (Fig. 1). This hormone-induced adipogenesis was significantly inhibited by treatment with SP (50 μg/mL) or SPP (≥10 μg/mL).

Effects on gene expression and protein synthesis

In RT-PCR analysis, mRNA expression of adipogenesis-specific leptin and Acrp30 (adipocyte-derived cytokines) was markedly increased by insulin inducer (Fig. 2A). SP at 1 μg/mL remarkably suppressed leptin expression, but was ineffective on Acrp30. However, a high concentration (50 μg/mL) of SP inhibited the expression of both leptin and Acrp30 mRNA. In comparison, SPP (1–50 μg/mL) suppressed the expression of both leptin and Acrp30, indicative of a higher activity than SP at low concentrations. Western blot analysis revealed markedly enhanced production of PPAR-γ and Acrp30 (adipogenesis-related proteins) in the presence of insulin (Fig. 2B). However, SP lowered both PPAR-γ and Acrp30 synthesis, in which the inhibition was specific for Acrp30. SPP produced a similar inhibitory pattern, although SPP was superior to SP.

Effects on daily feed consumption

The daily consumption of normal feed was 3.7–4.1 g/mouse (Fig. 3). However, the consumption of the D12451 HFD remarkably decreased by 20% (2.8–3.5 g/mouse), which may be due to the reduced palatability to a high content of lard (20.69%). By comparison, SP or SPP supplementation did not markedly affect the HFD intake, although there were weekly fluctuations for 8 weeks.

Effects on body weight

Mice fed a HFD displayed higher body weight gain than the control animals fed the D12450B normal diet (Fig. 4A, B). However, SP supplementation attenuated the body weight increase from 10 days after the initiation of feeding, leading to 35% lower body weights at 8 weeks in the 3.0%

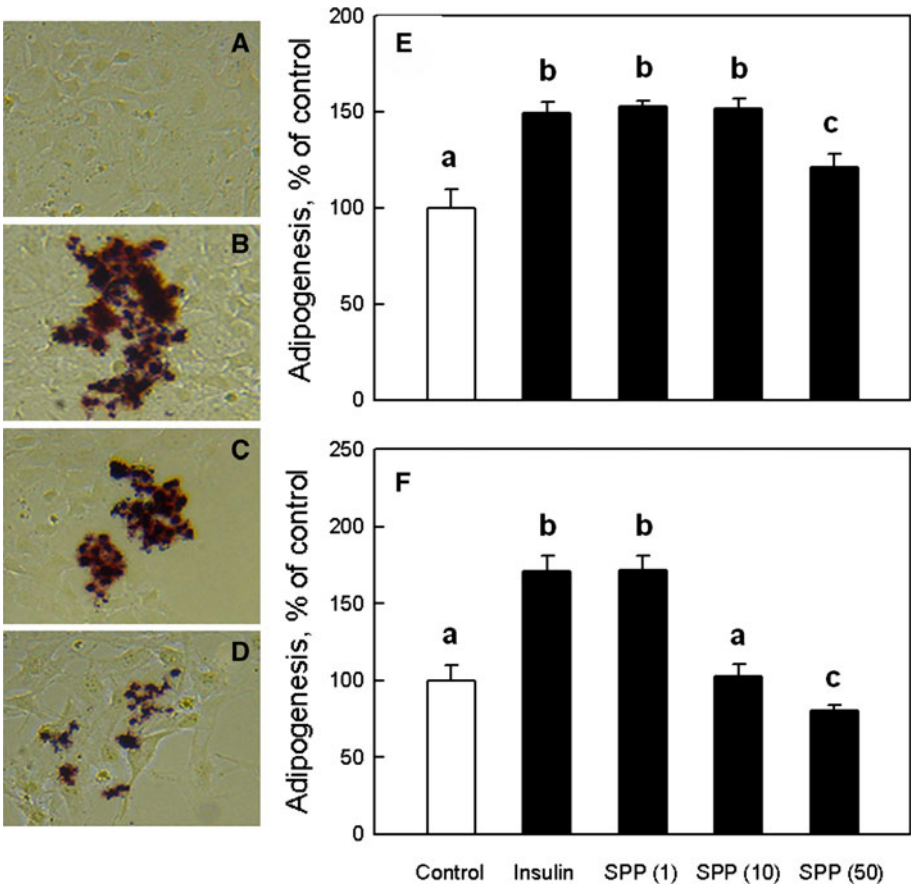

Fig. 1 Effects of silk peptide (SP) and silkworm pupa peptide (SPP) on the hormone-induced adipogenesis. 3T3-L1 cells were treated with various concentrations (1, 10, or 50 μg/mL) of SP or SPP and a hormone sensitizer (insulin, 5 μg/mL) for 4 days, and then lipid was quantified with adipogenesis kit after staining with Oil red O. Pictures **A–D** were taken just before extracting the Oil red O using IX51 inverted microscope system (40X, Olympus, Tokyo, Japan). **A** control; **B** insulin alone; **C** insulin + SP (50 μg/mL); **D** insulin + SPP (50 μg/mL); **E** insulin + SP (1–50 μg/mL); **F** insulin + SPP (1–50 μg/mL). Values with the same superscript letter (*a–c*) are not significantly different ($P < 0.05$)

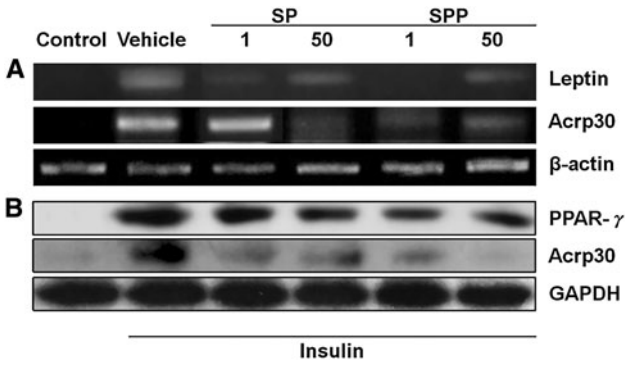

Fig. 2 Effects of silk peptide (SP) and silkworm pupa peptide (SPP) on the hormone-induced mRNA expression of adipogenesis-derived genes, leptin and Acrp30 (**A**) and production of adipogenesis-related proteins, peroxisome proliferator-activated receptor-γ (PPAR-γ), and Acrp30 (**B**). 3T3-L1 cells were treated with 1 or 50 μg/mL of SP or SPP and insulin (5 μg/mL) for 4 days, and then mRNA and proteins were analyzed by RT-PCR and Western blotting using optimal primers and specific antibodies, respectively

SP-fed group. Notably, SPP exhibited higher body weight-suppressive efficacy than SP, resulting in a 55% decrease after the 8-week feeding of 3.0% SPP.

Effects on fat weight

Weights of epididymal and mesenteric adipose tissue increased to 245% and 165% of control group, respectively, after the 8-week feeding of HFD (Fig. 4C, D). This HFD-induced increase in fat tissues was greatly suppressed by supplementation of the diet by 0.3–3.0% SP or SPP.

Effects on abdominal fat distribution

MRI analysis of abdominal adipose tissues revealed a 30–33% occupation in normal mice and an increase to 47–52% in the HFD-fed group (Fig. 5). Such an increase in the abdominal distribution of fat tissue was lowered by SP and SPP supplements in a concentration-dependent manner, leading to a normal level in the 3.0% SPP group (Fig. 5C, D).

Effects on adipocyte size

The size of epididymal adipocytes increased by 70% in mice fed HFD compared with cells in the control group (Fig. 6; Supplementary Fig. 1). The increased cell size was inhibited by supplementation with 1.0–3.0% of SP or SPP, resulting in suppression to a normal level at 3.0% of either supplement.

Effects on hepatic lipid accumulation

Livers of mice fed HFD for 8 weeks revealed many lipid droplets (Supplementary Fig. 2). Steatosis was attenuated

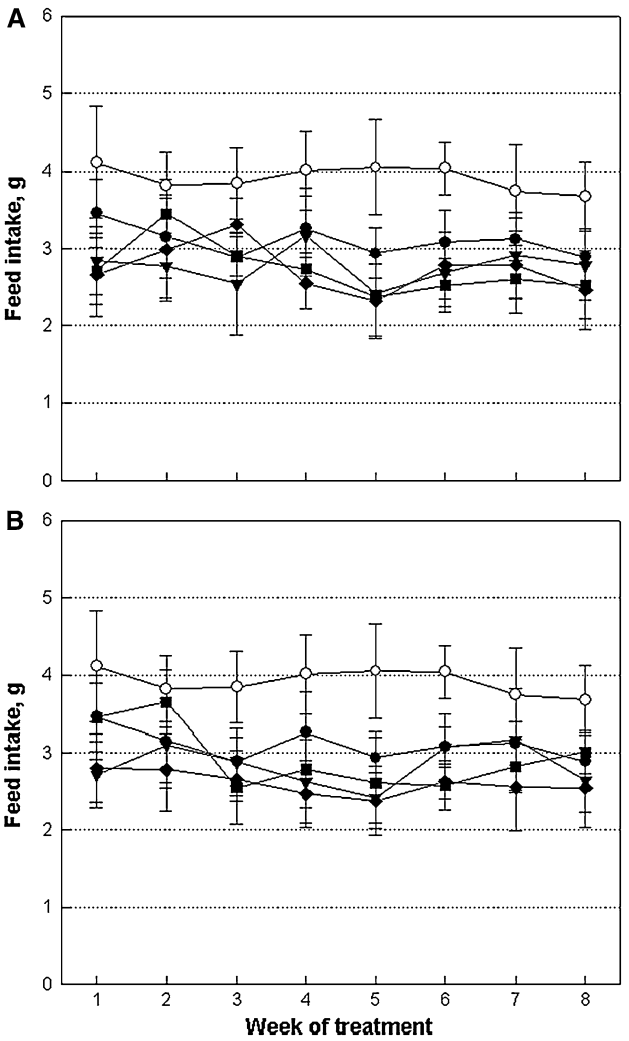

Fig. 3 Daily consumption (g/mouse) of a high-fat diet (HFD) containing silk peptide (**A**) or silkworm pupa peptide (**B**). Symbols: *circle*, normal diet; *filled circle*, HFD alone; *filled inverted triangle*, HFD + 0.3% peptides; *filled square*, HFD + 1.0% peptides; *filled diamond*, HFD + 3.0% peptides. Values with the same superscript letter are not significantly different ($P < 0.05$)

by supplementation with SP or SPP, in which 3.0% SPP fully resolved the fatty liver.

Effects on blood parameters

In the blood of HFD-fed mice, significantly higher leptin and Acrp30 levels were detected than in control animals (Table 2). The increases in blood leptin and Acrp30 were attenuated by SP and SPP, although SPP was superior to SP at all concentrations. HFD also enhanced blood TG and cholesterol, in which TG was reversed by SP (0.3 and 3.0%) and SPP (0.3–3.0%), although cholesterol was significantly reduced by only 1.0% SPP. Notably, HFD increased blood glucose level, but it was significantly recovered by 1.0% SP and 1.0–3.0% SPP.

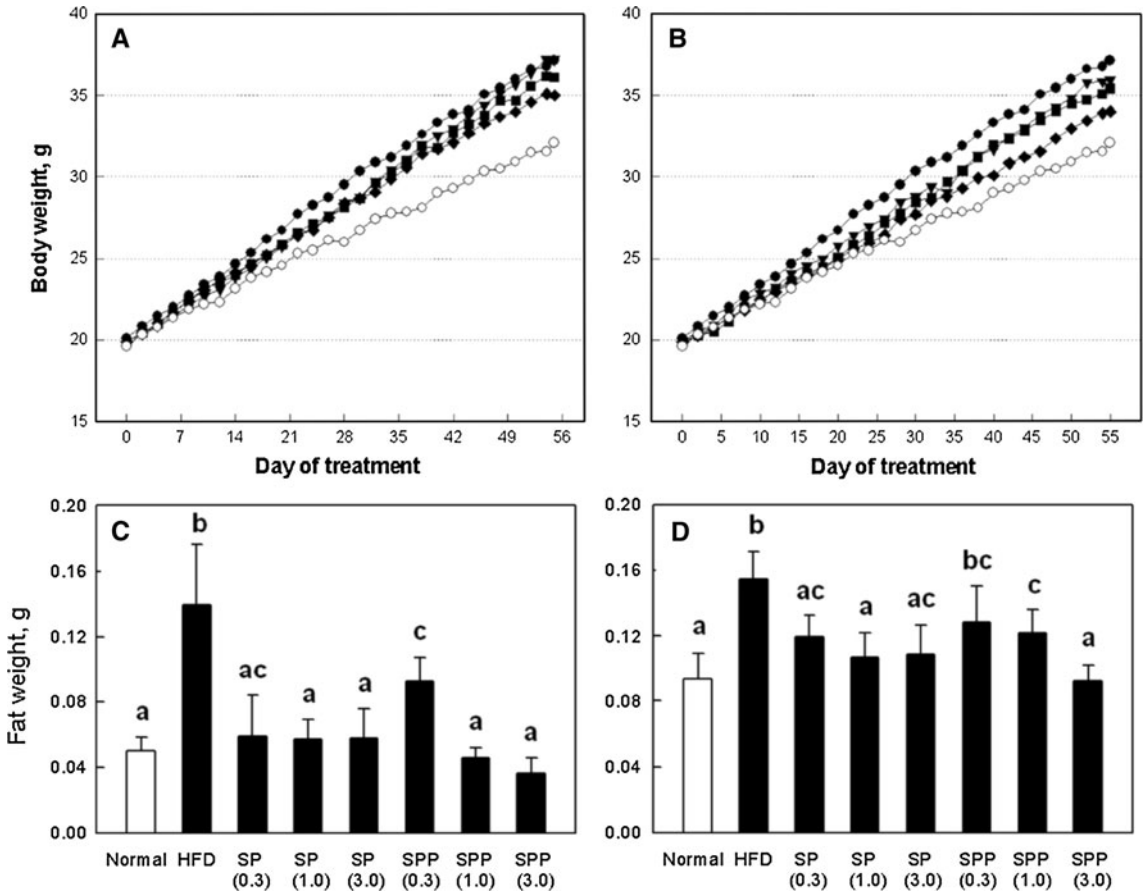

Fig. 4 Effects of silk peptide (SP, **A** and **C**) or silkworm pupa peptide (SPP, **B** and **D**) on the body weight gain (**A** and **B**) and epididymal (**C**) and mesenteric (**D**) fat weights of mice fed a high-fat diet (HFD). Symbols: *circle*, normal diet; *filled circle*, HFD alone; *filled inverted triangle*, HFD + 0.3% peptides; *filled square*, HFD + 1.0% peptides; *filled diamond*, HFD + 3.0% peptides. Values with the same superscript letter (*a–c*) are not significantly different ($P < 0.05$)

Discussion

The present study confirmed that SP and SPP supplementation attenuate body weight gain of HFD-fed animals by decreasing the accumulation of body fats and the size of adipocytes. These results are due to SP- and SPP-mediated inhibited adipogenesis of adipocytes by blocking adipogenic gene expression and protein synthesis.

Obesity is a major risk factor of degenerative disorders such as diabetes mellitus, hypertension, cardiovascular diseases, and inflammatory diseases [33]. Interestingly, the distribution of body fats differently affects disease morbidity [34]; especially, abdominal adipose tissue decreases insulin tolerance, while it enhances the incidence of atherosclerosis and cardiovascular diseases [35, 36].

Since body fats are synthesized in mature adipocytes through processes of differentiation and maturation of preadipocytes, many investigations have focused on adipogenic signal transduction to develop anti-obesity materials. Preadipocytes differentiate and mature in response to hormones including insulin via expression of adipogenic genes such as PPAR-γ, CCAAT/enhancer-binding proteins, and sterol regulatory element-binding proteins and produce TG [37, 38]. In the present study, 3T3-L1 preadipocytes induced by insulin (5 μg/mL), dexamethasone (0.25 μM), and IBMX (0.5 mM) also produced more TG (50–70% increase) than non-stimulated cells.

Whereas SP significantly suppressed maturation and adipogenesis of 3T3-L1 cells at 50 μg/mL, a concentration inducing mild cytotoxicity (10–100 μg/mL), SPP produced similar effects at concentrations as low as 10 μg/mL, which were much lower than its cytotoxic concentration (1,000 μg/mL). Analyses of transcriptional factors have demonstrated that the anti-adipogenic effects of SP and SPP are due to their inhibition of gene expression of leptin [31, 32] and Acrp30, as well as the synthesis of PPAR-γ and Acrp30 proteins. Interestingly, SPP was superior to SP in the inhibition of cell signaling, differentiation, maturation, and adipogenesis. Similarly, a study demonstrated that grape seed dregs inhibit PPAR-γ protein synthesis and following TG accumulation at 100 μg/mL [28]. By comparison, SPP presently suppressed PPAR-γ production and

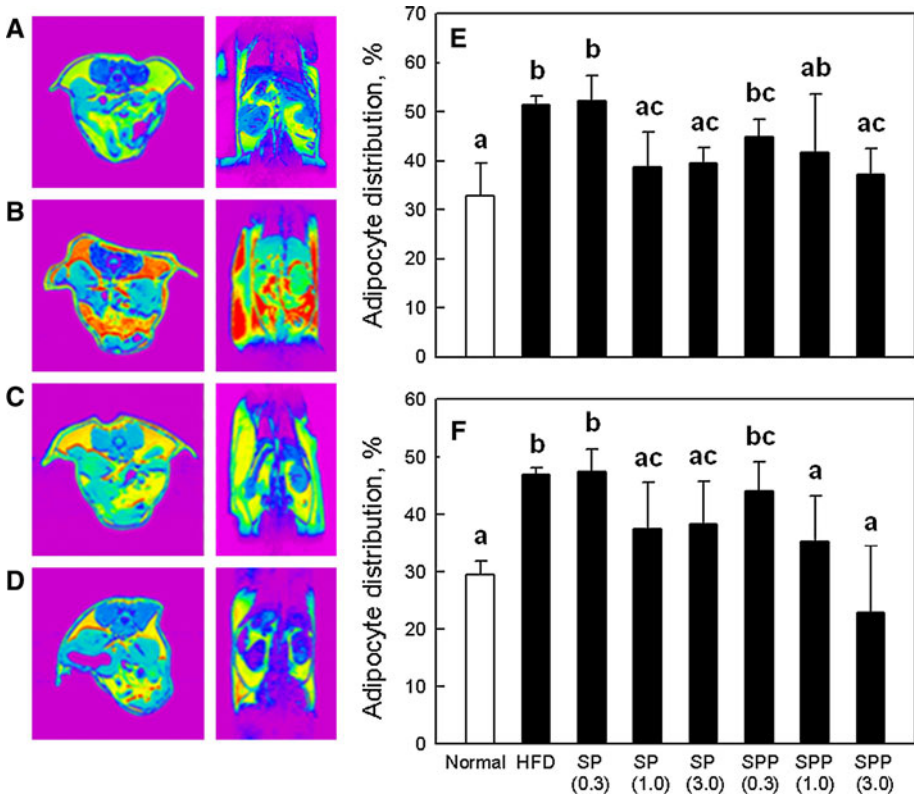

Fig. 5 Effects of silk peptide (SP) or silkworm pupa peptide (SPP) on the distribution of abdominal adipose tissue of mice fed a high-fat diet (HFD). **A** Normal diet; **B** HFD alone; **C** HFD + 3.0% SP; **D** HFD + 3.0% SPP. Figures **E** and **F** display transverse and coronal sections of magnetic resonance images as shown *left* and *right* panels in **A**–**D**, respectively. Values with the same superscript letter (*a*–*c*) are not significantly different ($P < 0.05$)

TG accumulation at 1 and 10 μg/mL, respectively, which were much lower than the effective concentration of grape seed dregs.

The body weight gain of mice fed the D12451 HFD markedly increased in spite of the reduced consumption of HFD (about 20%), compared to normal diet. However, supplementation of SP and SPP in HFD for 8 weeks reduced body weight gain; especially, 3.0% SPP exerted a 55% reversing effect. Such body weight-controlling effects of SP and SPP likely reflect decrease in body fat weights. Presently, the reduction of fat weights was due to the lowered amount of fat tissue as well as the decreased size of adipocytes, which were confirmed from MRI analysis on the abdominal fat distribution, direct measurement of fat weights, and image analysis of adipocytes. Notably, SP and SPP significantly reduced the epididymal and mesenteric adipose tissues, implying that they can display a beneficial effect on abdominal fats, which induce metabolic syndrome, rather than subcutaneous fats [35].

In our previous study [26], silk amino acids (SAA-1) and silkworm pupa amino acids (SAA-3) reduced the body weight gain of resting animals. Furthermore, SAA-3 facilitated exercise-induced reduction of body weight gain, leading us to a conclusion that intake of SAA-3 during exercise synergistically decreases the body weight gain. In the present study, it was confirmed that peptides (SP and SPP), prior to complete degradation to amino acids, also

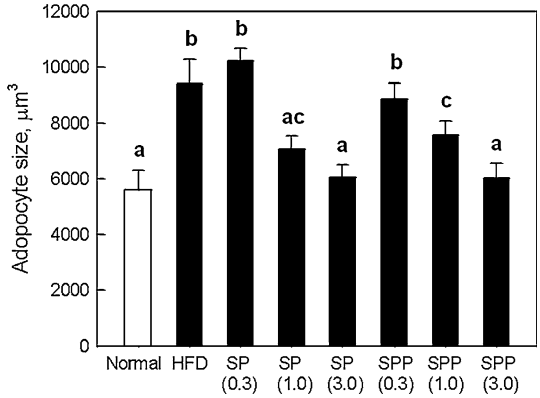

Fig. 6 Effects of silk peptide (SP) or silkworm pupa peptide (SPP) on the size of epididymal adipocytes of mice fed a high-fat diet (HFD). Values with the same superscript letter (*a*–*c*) are not significantly different ($P < 0.05$)

attenuate body weight gain of mice with obesity induced by HFD. It is of interest to note previous reports that SAA greatly enhances stamina (forced swimming time) in animals and humans not only by blocking tissue (muscle) damages, but also by preserving energy sources, in spite of marked body weight-reducing activity [26, 27]. Also, SAA-1 significantly improves physical function of Parkinson's disease model rats [39], and SP recovers cognitive (learning and memory) function of facilitated brain-aging model animals [40].

Table 2 Effects of silk peptide (SP) or silkworm pupa peptide (SPP) on the blood concentration of markers of adipogenesis, lipids, glucose, and hepatic injury of mice fed a high-fat diet (HFD) after treatment for 8 weeks

Treatment (%)	Leptin (ng/mL)	Acrp30 (ng/mL)	Triglycerides (mg/dL)	Cholesterol (mg/dL)	Glucose (mg/dL)
Normal	3.1 ± 0.4^{a}	133.0 ± 11.2^{a}	84.0 ± 12.7^{a}	95.8 ± 26.8^{a}	113.0 ± 13.9^{a}
HFD	5.2 ± 1.1^{b}	230.1 ± 25.2^{b}	154.3 ± 12.5^{b}	117.3 ± 12.1^{b}	159.7 ± 14.1^{b}
+SP (0.3)	4.7 ± 2.1^{ab}	223.4 ± 30.1^{bc}	124.7 ± 11.6^{c}	112.7 ± 4.0^{b}	141.7 ± 16.8^{b}
+SP (1.0)	5.6 ± 2.3^{b}	216.0 ± 31.1^{c}	140.0 ± 23.5^{bc}	108.0 ± 5.3^{ab}	118.8 ± 15.8^{a}
+SP (3.0)	3.5 ± 0.3^{a}	90.5 ± 18.6^{d}	131.2 ± 9.8^{c}	115.3 ± 14.5^{b}	134.0 ± 12.6^{c}
+SPP (0.3)	4.6 ± 0.8^{b}	194.5 ± 24.8^{c}	123.0 ± 12.1^{c}	110.0 ± 10.5^{ab}	142.7 ± 18.0^{b}
+SPP (1.0)	4.3 ± 1.2^{ab}	185.3 ± 31.9^{c}	120.8 ± 22.4^{cd}	106.3 ± 3.6^{a}	118.3 ± 13.5^{a}
+SPP (3.0)	3.0 ± 0.9^{a}	152.5 ± 48.6^{ac}	94.3 ± 13.6^{ad}	108.3 ± 9.3^{ab}	129.1 ± 13.3^{a}

Values with the same superscript letter (a–d) are not significantly different ($P < 0.05$)

Presently, the long-term application of an HFD increased blood TG, cholesterol, and glucose; these changes reflected changes in their regulatory hormones, Acrp30 and leptin. In addition, HFD caused hepatic accumulation of TG, as confirmed by microscopic examination. Interestingly, SP and SPP reversed the increased blood parameters of lipid metabolism and related hormones as well as glucose, and the lipid deposition in the liver. Such beneficial effects of silk products (proteins, peptides, and amino acids) on blood glucose and lipids have been reported by many investigators including our group [20–25]. It was proposed that the lipid- and/or glucose-controlling effects of silk protein hydrolysates were due to their up-regulating activities on the leptin and insulin secretion [21, 22]. In this study, we present additional molecular signaling factors Acrp30 and PPAR-γ for their anti-obesity and anti-diabetic activities.

In our repeated-dose toxicity tests, SAA and SP did not induce adverse effects on general physical functions and on the immune system [41]. Taken together, it is suggested that SP and SPP inhibit adipogenesis by blocking adipogenic gene expression and protein synthesis, resulting in lowered body weight gain, and that they could be promising adverse-free candidates for the improvement of dietary obesity.

Acknowledgments This work was supported by Priority Research Centers Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2009-0094035).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Hill JO, Peters JC, Wyatt HR (2007) The Role of public policy in treating the epidemic of global obesity. Clin Pharmacol Ther 81: 772–775
2. Manson JE, Willet WC, Stamfer MJ, Colditz GA, Hunter DJ, Hankinson SE, Hennekens CH, Speizer FE (1995) Body weight and mortality among woman. N Engl J Med 333:677–685
3. Hill JO, Holly RW, Reed GW, Peters JC (2003) Obesity and the environment: where do we go from here? Science 299:853–855
4. Hart RW, Keenan KP, Turturro A, Abdo KM, Leakey J, Lyn-Cook L (1995) Calorie restriction and toxicology. Fund Appl Toxicol 25:184–195
5. Milagro FI, Campion J, Martinez A (2006) Weight gain induce by high-fat feeding involves increased liver oxidation stress. Obes Res 14:1118–1123
6. Colditz GA, Willett WC, Rotnitzky A, Manson JE (1995) Weight gain as a risk factor for clinical diabetes mellitus in women. Ann Intern Med 122:481–486
7. Rothwell NJ, Stock MJ (1984) The development of obesity in animals; the role of dietary factors. Clin Endocrinol Metab 13: 437–449
8. Lee JJ, Choi HS, Jeong E, Choi BD, Lee MY (2006) Effect of meal pattern on lipogenesis and lipogenic enzyme activity in rat adipose tissue fed high fat diet. J Korean Soc Food Sci Nutr 35:335–343
9. Beaumont JL, Carlson LA, Cooper GR, Fejfar Z, Fredrickson DS, Strasser T (1970) Classification of hyperlipidemia and hyperlipoproteinemia. Bull World Health Organ 43:891–915
10. Ahren B, Scheurink AJ (1998) Marked hyperleptinemia after high-fat diet associated with severe glucose intolerance in mice. Eur J Endocrinol 139:461–467
11. Handa T, Yamaguchi K, Sono Y, Yazawa K (2005) Effects of fenugreek seed extract in obese mice fed a high-fat diet. Biosci Biotechnol Biochem 69:1186–1188
12. Woods SC, Seeley RJ, Rushing PA, D'Alessio D, Tso P (2003) A controlled high-fat diet induces an obese syndrome in rats. J Nutr 133:1081–1087
13. Vasselli JR, Weindruch R (2005) Intentional weight loss reduces mortality rate in a rodent model of dietary obesity. Obes Res 13:693–702
14. Murphy TA, Lerch SC (1994) Effects of restricted feeding of growing steers on performance, carcass characteristics, and composition. J Anim Sci 72:2497–2507
15. Cho YM, Park D, Jeon JH, Jang MJ, Kim JJ, Kim JW, Ji HJ, Kim CH, Baek S, Hwang SY, Kim G, Kim YB (2007) Effect of feed restriction in modeling of dietary obesity. Lab Anim Res 23: 427–434
16. Surwit RS, Feinglos MN, Rodin J, Sutherland A, Petro AE, Opara EC, Kuhn CM, Rebuffe-Serive M (1995) Differential effects of fat and sucrose on the development of obesity and diabetes in C57BL/6J and A/J mice. Metabolism 44:645–651

17. Lin S, Thomas TC, Storlien LH, Huang XF (2000) Development of high fat diet-induced obesity and leptin resistance in C57Bl/6J mice. Int J Obes Relat Metab Disord 24:639–646
18. Reeves PG (1997) Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 127(5 Suppl): 838S–841S
19. Kishino E, Ito T, Fujita K, Kiuchi Y (2006) A mixture of the *Salacia reticulata* (Kotala himbutu) aqueous extract and cyclodextrin reduces the accumulation of visceral fat mass in mice and rats with high-fat diet-induced obesity. J Nutr 136:433–439
20. Shin M, Park M, Youn M, Lee Y, Nam M, Park I, Jeong Y (2006) Effects of silk protein hydrolysates on blood glucose and serum lipid in db/db diabetic mice. J Korean Soc Food Sci Nutr 35: 1343–1348
21. Lee Y, Park M, Choi J, Kim J, Nam M, Jeong Y (2007) Effects of silk protein hydrolysates on blood glucose level, serum insulin and leptin secretion in OLEFT rats. J Korean Soc Food Sci Nutr 36:703–707
22. Jung EY, Lee HS, Lee HJ, Kim JM, Lee KW, Suh HJ (2010) Feeding silk protein hydrolysates to C57BL/KsJ-db/db mice improves blood glucose and lipid profiles. Nutr Res 30:783–790
23. Kim TM, Ryu JM, Seo IK, Lee KM, Yeon S, Kang S, Hwang SY, Kim YB (2008) Effects of red ginseng powder and silk peptide on hypercholesterolemia and atherosclerosis in rabbits. Lab Anim Res 24:67–75
24. Kato N, Sato S, Yamanaka A, Yamada H, Fuwa N, Nomura M (1998) Silk protein, sericin, inhibits lipid peroxidation and tyrosinase activity. Biosci Biotechnol Biochem 62:145–147
25. Zhaorigetu S, Yanaka N, Sasaki M, Watanabe H, Kato N (2003) Silk protein, sericin, suppresses DMBA-TPA-induced mouse skin tumorigenesis by reducing oxidative stress, inflammatory responses and endogenous tumor promoter TNF-α. Oncol Rep 10:537–543
26. Shin S, Park D, Yeon S, Jeon JH, Kim TK, Joo SS, Lim WT, Lee JY, Kim YB (2009) Stamina-enhancing effects of silk amino acid preparations in mice. Lab Anim Res 25:127–134
27. Shin S, Yeon S, Park D, Oh J, Kang H, Kim S, Joo SS, Lim WT, Lee JY, Choi KC, Kim KY, Kim SU, Kim JC, Kim YB (2010) Silk amino acids improve physical stamina and male reproductive function of mice. Biol Pharm Bull 33:273–278
28. Choi Y, Lee SM, Kim Y, Jeon G, Sung J, Jeong HS, Lee J (2010) Defatted grape seed extracts suppress adipogenesis in 3T3–L1 preadipocytes. J Korean Soc Food Sci Nutr 39:927–931
29. Joo SS, Park D, Shin S, Jeon JH, Kim TK, Choi YJ, Lee SH, Kim JS, Park SK, Hwang BY, Lee DI, Kim Y-B (2010) Anti-allergic effects and mechanisms of action of the ethanolic extract of *Angelica gigas* in dinitrofluorobenzene-induced inflammation models. Environ Toxicol Pharmacol 30:127–133
30. Hwang JH, Kim DW, Jo EJ, Kim YK, Jo YS, Park JH, Yoo SK, Park MK, Kwak TH, Kho YL, Han J, Choi HS, Lee SH, Kim JM, Lee I, Kyung T, Jang C, Chung J, Kweon GR, Shong M (2009) Pharmacological stimulation of NADH oxidation ameliorates obesity and related phenotypes in mice. Diabetes 58:965–974
31. Levy JR, Lesko J, Krieg RJ Jr, Robert RA, Stevens W (2000) Leptin responses to glucose infusions in obesity-prone rats. Am J Physiol Endocrinol Metab 279:E1088–E1096
32. Mercer JG, Hoggard N, Morgan PJ (2000) Leptin and obesity; the story so far and it therapeutic implication. CNS Drugs 14: 413–424
33. Visscher TL, Seidell JC (2001) The public health impact of obesity. Annu Rev Public Health 22:355–375
34. Prineas RJ, Folsom AR, Kaye SA (1993) Central adiposity and increased risk of coronary disease mortality in older women. Ann Epidemiol 3:35–41
35. Despres JP (1998) The insulin resistance-dyslipidemic syndrome of visceral obesity: effect on patient's risk. Obes Res 6:8S–17S
36. Niklas BJ, Penninx BW, Ryan AS, Berma DM, Lynch NA, Dennis KE (2003) Visceral adipose tissue cutoffs associated with metabolic risk factors for coronary heart disease in women. Diabetes Care 26:1413–1420
37. Morrison RF, Farmer SR (2000) Hormonal signaling and transcriptional control of adipocyte differentiation. J Nutr 130: 3116S–3121S
38. Ntambi JM, Kim YC (2000) Adipocyte differentiation and gene expression. J Nutr 130:3122S–3126S
39. Kim TK, Park D, Yeon S, Lee SH, Choi YJ, Bae DK, Yang YH, Yang G, Joo SS, Lim WT, Lee JY, Lee J, Jeong HS, Hwang SY, Kim YB (2011) Tyrosine-fortified silk amino acids improve physical function of Parkinson's disease rats. Food Sci Biotechnol 20:79–84
40. Park D, Lee SH, Choi YJ, Bae DK, Yang YH, Yang G, Kim TK, Yeon S, Hwang SY, Joo SS, Kim YB (2011) Improving effect of silk peptides on the cognitive function of rats with aging brain facilitated by $_{D}$-galactose. Biomol Ther 19:224–230
41. Ryu JM, Kim TM, Seo IK, Yeon S, Lim WT, Lee JY, Hwang SY, O NG, Song J, Lee J, Kim YB (2008) Effect of repeated administration of silk peptide on the immune system of rats. Lab Anim Res 24:361–369